Dichelesthium oblongum (Copepoda: Dichelesthiidae) infestation in wild-caught Atlantic sturgeon, Acipenser oxyrinchus oxyrinchus

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Atlantic sturgeon were sampled in autumn 2007 and 2008 along the New York Bight. The fish were examined for the presence of external parasites, and blood, fin, and opercula biopsies were collected for subsequent serum analysis and histopathological evaluation. Dichelesthium oblongum, a parasitic copepod, was observed on 93% of the sturgeon sampled (77 out of 83) between Rockaway and Jones Beaches, NY, and Sandy Hook, NJ. During the course of the examinations, grossly visible lesions associated with the attachment and feeding of juvenile stages of D. oblongum were noted on the operculum, pectoral, dorsal, and anal fins of infested fish. Significant differences were observed between the level of infestation and serum chemistry across sampling sites, such that the most heavily infested fish were caught off Jones Beach (15.8 $\pm$ 2.79 lice fish^{-1}), higher infestations than at Rockaway Beach (8.86 $\pm$ 0.89 lice fish^{-1}) and Sandy Hook (5.31 $\pm$ 1.40 lice fish^{-1}). Animals from Jones Beach also indicated ion loading (i.e. sodium, calcium, and magnesium), possibly as a result of stress or water loss through D. oblongum infestation compromising the epithelial barrier. The interaction of the environment and parasite with host life-history characteristics are discussed in terms of their ecological significance to this threatened fish species.

Keywords: Atlantic sturgeon, copepod, Dichelesthium oblongum, histopathology, parasite, serum chemistry.

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Introduction

Atlantic sturgeon, Acipenser oxyrinchus oxyrinchus, are large ($\leq$300 kg) anadromous fish which make extensive coastal movements, spending several years in marine waters. They are a long-lived ($\sim$60 years), late-maturing (8–17 years) species, and mature fish do not spawn every year. Spawning intervals range from 1 to 5 years for males (Smith, 1985; Collins et al., 2000) and from 2 to 5 years for females (Vladykov and Greeley, 1963; Van Eenennaam and Doroshov, 1998; Stevenson and Secor, 1999). These factors make Atlantic sturgeon particularly susceptible to fisheries overharvest and environmental change. Furthermore, severe population declines over the past century have led to the implementation of a fisheries moratorium in 1998 (Waldman and Wirgin, 1998; ASSRT, 2007). The largest remaining population in the United States is currently located in the Hudson River, NY, although it is in decline (ASSRT, 2007). Although the general life cycle of Atlantic sturgeon is well known, information gaps exist regarding their movements and juvenile/immature habitat usage upon leaving their natal rivers, homeostatic responses to these differing osmotic environments, and their physiological consequences in terms of stress and disease.

Dichelesthium oblongum is a marine parasitic copepod known to infest the gills of European and North American acipenserids (Appy and Dadswell, 1978; Bauer et al., 2002). It is predicted that D. oblongum contains 10 life stages: nauplii I and II, infective copepodid, parasitic copepodids I–VI, and adult. To date, only the adult parasites on the gills of sturgeon have been described in the literature (Kabata and Khodorevsky, 1977; Kabata, 1979; Bauer et al., 2002), and one short report on the infective copepodid stage can be found. Despite these reports on sturgeon hosts, virtually nothing is known of the general biology, ecology, routes of infestation, epidemiology, and host–parasite interactions of the genus. In comparison, other siphonostomatoid copepods are better studied and have significant effects on their hosts. The parasitic copepods of this group have been associated with morbidity and mortality in marine fish and have resulted in epizootics in anadromous fish stocks (reviewed in Costello, 2006; Wagner et al., 2008). Of most recent concern are the effects of the caligid copepod Lepeophtheirus salmonis on declining and threatened populations of Pacific salmon and Atlantic sea trout (Pike and Wadsworth, 1999; Costello, 2006).

The current investigation was undertaken to determine whether any pathological effects were associated with D. oblongum infestation in the Atlantic sturgeon that aggregate near the Hudson River. Because of the threatened population status of Atlantic sturgeon, we used non-invasive methods of sampling, such as skin biopsies at sites of infestation, and blood collection for blood-chemistry analysis to determine the patho-physiological consequences of D. oblongum infestation.
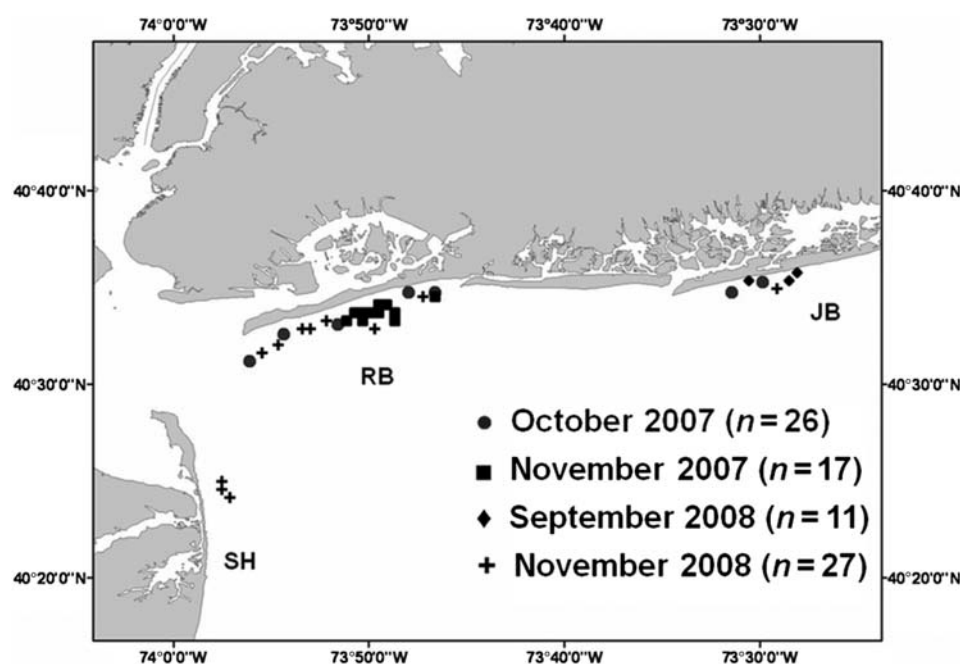
Figure 1. Atlantic sturgeon sampling area from autumn 2007 until autumn 2008. JB, Jones Beach; RB, Rockaway Beach; and SH, Sandy Hook, NJ.

Material and methods
Fish sampling
Juvenile Atlantic sturgeon were captured by bottom trawl, an 80′ otter trawl, aboard the RV "Seawolf" within juvenile/immature ocean habitat outside Jones Beach, NY, Rockaway, NY, and Sandy Hook, NJ (Figure 1). Tows were conducted for 10–20 min to minimize damage and/or stress-related impacts on the fish. Four collection trips were conducted in 2007/2008, each lasting several days, starting 16 October 2007, 15 November 2007, 15 September 2008, and 21 November 2008. Upon capture, all sturgeon were identified to species, measured for total and fork length (cm), weighed (kg), were scanned for ectoparasites (collected in 10% neutral-buffered formalin), and had blood and skin biopsy samples taken. Those animals not immediately sampled were maintained in live wells for short periods (0–60 min). Following these procedures and tagging, fish were released (a 15–20-min procedure in total). Condition factor (CF) was calculated for each fish using fork length (L; cm) and weight (W; g) in the equation $CF = (W/L^3) \times 100$.

Serum analysis
To minimize any manipulation-induced changes in serum chemistry, blood samples were collected shortly after the fish were brought aboard the vessel, before parasitological examination. Blood samples were taken from the caudal vein (ca. 1–3 ml) with a 5-cc syringe and 18 G-1 needles. Blood was then allowed to clot for up to 6 h ($<15°C$), and serum was collected after a 5–10-min centrifuge at 4000g. Serum samples were immediately frozen at $-80°C$ until analysed. Serum samples were submitted to the Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, and serum panel analyses conducted using a Hitachi 917 blood chemistry analyser. Anion gap was calculated as the sum of cations minus the sum of anions $[(Na^+ + K^+) - (Cl^- + HCO_3^-)]$.

Histological analysis
Biopsies of sites of infestation from the fins and opercula were fixed in 10% neutral-buffered formalin for later histological processing. Tissues were embedded in paraffin, and 6 μm sections cut and fixed on slides, then stained with haematoxylin and eosin (Luna, 1968). In addition, gram-stains for bacteria were also applied to representative sections. Histological sections were examined under a Nikon TE2000 using brightfield illumination. Images were captured using an Insight digital camera and processed using the Spot Advanced software, version 3.5 (Diagnostic Instruments Inc.).

Statistical analysis
All data were analysed using SYSTAT version 3.1. Data were tested for normality, but when normality tests failed, a square-root transformation was applied to the data. If data still did not conform to a normal distribution, they underwent Kruskal–Wallis one-way ANOVA on ranks and pairwise comparison using Dunn's method. All normal data were analysed using a one-way ANOVA and pairwise comparison using the Holm–Sidak method. Significant differences were determined based on $p < 0.05$. Data are expressed as mean $\pm$ SEM, unless otherwise denoted.

Results
General parasitology
Of the 83 fish examined for external parasites, the prevalence was 0.02% for an unknown species of isopod (2 fish out of 83), 30% for *Caligus elongatus* (25 out of 83), 70% for *Nitzschia* spp. (58 out of 83), and 93% for *D. oblongum* (77 out of 83). Abundances of *D. oblongum* ranged from 0 to 40 per sturgeon. The parasites present were mainly gravid females (size range 7–17 mm; mean size 11.7 $\pm$ 1.3 mm) that were attached to opercular cavities and occasionally on the gill or gill arch (404 of 601, i.e. 67%). Another 107 *D. oblongum*, all of which were juvenile stages, were

observed attached to other external body surfaces (i.e. the mouth and the fins). *Caligus elongatus* infestations were always found on the lateral surfaces of the body and usually consisted of a single parasite, but reached a maximum of four on occasion. Whereas monogenean, *Nitzschia* spp., infestations ranged from 1 to 13 parasites, although rarely (15%) consisted of >4, and were mainly on the gills, with fewer on and around the mouth.

Differences were observed between the level of infestation and the percentage of gravid female *D. oblongum* across sampling sites, such that the most heavily infested sturgeon were caught off Jones Beach (15.8 ± 2.79 lice fish^{-1}) and infestation levels were less at Rockaway Beach (8.86 ± 0.89 lice fish^{-1}) and Sandy Hook (5.31 ± 1.40 lice fish^{-1}; Figure 2a). There was also a significant positive correlation (Pearson correlation 0.83) between total

D. oblongum abundance and *D. oblongum* gravid female abundance, such that there was a much larger proportion of sturgeon from Jones Beach with high (six or more) gravid female abundance than at the other two sites. No significant differences were observed between sites for the other parasites observed, but *C. elongatus* were rarely found on fish sampled from Jones Beach.

Although Atlantic sturgeon were slightly larger at Jones Beach and displayed slightly lower CFs, these data were not significantly different between sites and did not account for the differences in parasite burden (Table 1; one-way ANOVA, Kruskal–Wallis). There was no correlation between size and level of infestation. Moreover, four Atlantic sturgeon outliers were not included in the analysis owing to their sizes being well outside 2 s.d. from the mean. These larger fish (>25 kg) also had variable levels of infestation (2–37 lice fish^{-1}) with a mean of 10.0 ± 13.8 (s.d.).

Gross and histopathology

During the course of external examination, macroscopic lesions associated with the attachment and feeding of *D. oblongum* were observed on Atlantic sturgeon (Figure 3). Sloughing of skin and haemorrhaging were also observed in some cases at the site of *Nitzschia* spp. attachment near the sturgeon mouth and on the gills. Lesions associated with juvenile *D. oblongum* (Figure 3a and b) consisted of focal areas of ulceration with a raised border. Lesions were generally large (i.e. diameter greater than the length of the parasite always; 7–12 mm), and often located on the operculum or at the base of fins. Petechial haemorrhaging was gross close to gravid female attachment within the opercular cavity (Figure 3c). Histologically, the lesions were characterized by necrosis or complete loss of both epidermis and dermis, areas of haemorrhage next to juvenile attachment (Figure 4b), and focal necrosis of the underlying musculature. Focal epidermal spongiosis was also observed near lesions where the epidermis was still intact when compared with the epidermis next to the lesion (Figure 4c and d). None of the histological sections selected for gram-staining showed signs of bacterial infection.

Serum chemistry

Similar to the level of *D. oblongum* infestation, significant differences were exhibited in serum chemistry across sampling sites. Bicarbonate and urea nitrogen levels were significantly lower in Atlantic sturgeon sampled from Jones Beach than at Rockaway Beach and Sandy Hook (Table 1). Conversely, anion gap was significantly greater in sturgeon sampled from Jones Beach than at Rockaway Beach and Sandy Hook (Table 1).
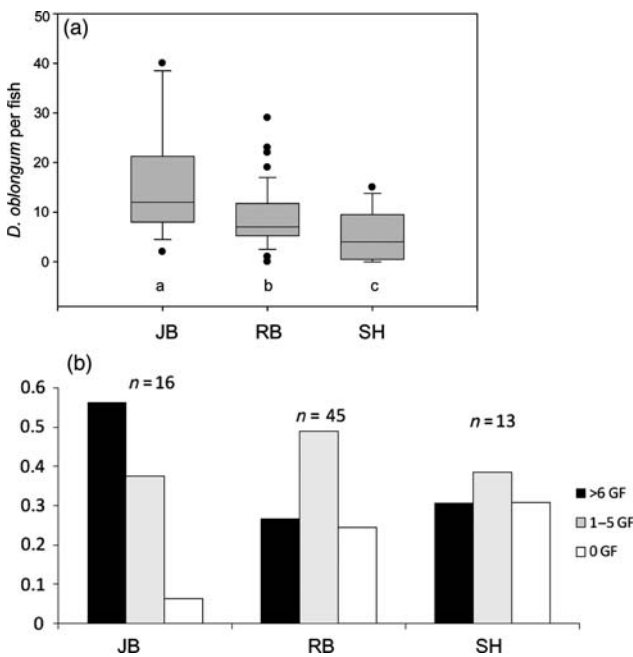

Figure 2. (a) Median *D. oblongum* (10th, 25th, 75th, and 90th percentiles, and outliers) levels of infestation, and (b) proportion of infested Atlantic sturgeon with 0, 1–5, and 6 or greater gravid females (GF), across three sampling sites in New York and New Jersey waters. JB, Jones Beach; RB, Rockaway Beach; SH, Sandy Hook, NJ. Letters denote significant difference between groups.

Table 1. Mean (± SEM) measurements of Atlantic sturgeon caught off New York and New Jersey, autumn 2007 and 2008.

Parameter	Jones Beach, NY (n = 14)	Rockaway Beach, NY (n = 44)	Sandy Hook, NJ (n = 13)
Total length (cm)	126.1 ± 8.11	114.0 ± 4.28	103.0 ± 3.11
Weight (kg)	8.63 ± 0.90	7.15 ± 0.55	6.30 ± 0.63
CF	0.827 ± 0.01	0.836 ± 0.01	0.834 ± 0.01
Bicarbonate (mEq l^{-1})	4.07 ± 0.30[a]	5.43 ± 0.27[b]	6.38 ± 0.29[b]
Chloride (mEq l^{-1})	140.9 ± 1.39	139.4 ± 0.762	140.5 ± 0.993
Anion Gap (mEq l^{-1})	28.5 ± 0.78[a]	23.7 ± 1.02[b]	20.8 ± 0.92[b]
Urea nitrogen (mg dl^{-1})	26.9 ± 1.14[a]	40.0 ± 1.18[b]	39.3 ± 1.98[b]
Glucose (mmol)	2.65 ± 0.34	2.49 ± 0.12	3.25 ± 0.22
Aspartate aminotransferase (units l^{-1})	302.9 ± 19.1	266.1 ± 14.3	294.1 ± 26.3
C. elongatus (lice fish^{-1})	0.07 ± 0.06	0.59 ± 0.14	0.69 ± 41

The anion gap is the sum of cations minus the sum of anions [($Na^+ + K^+$) − ($Cl^- + HCO_3^-$)], and CF was calculated for each fish using fork length (L; in cm) and weight (W; in g) from $CF = (WL^{-3}) \times 100$. Letters denote significant differences between groups.
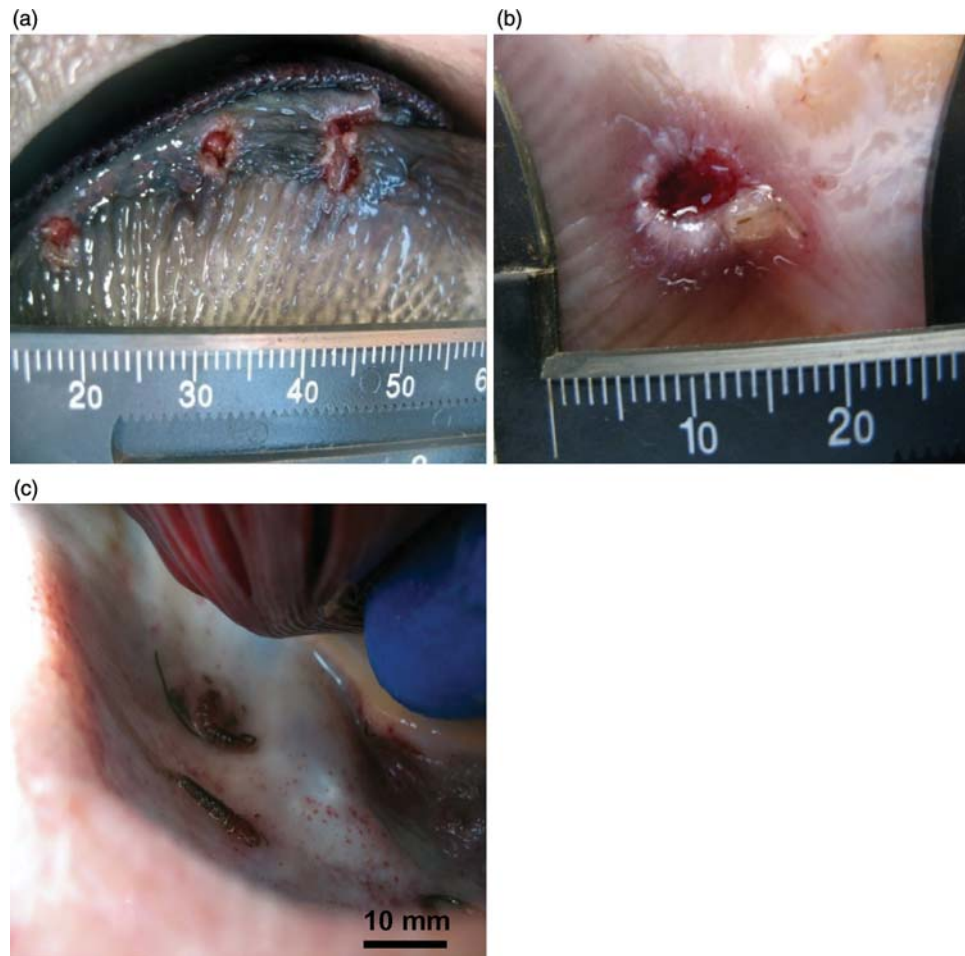
Figure 3. Juvenile *D. oblongum* infestation on (a) the operculum, (b) the base of the fins, and (c) gravid female copepods within the opercular cavity of Atlantic sturgeon [(a) and (b) scale bar in millimetres]. Note the petechial haemorrhaging of the host tissue associated with gravid female copepods in (c).

There were no significant differences observed in serum chloride, glucose, or aspartate aminotransferase (Table 1). All other serum ion data were significantly affected by the sampling site. Serum sodium (170.2 ± 1.55 mEq l^{-1}), phosphate (13.9 ± 0.06 mg dl^{-1}), magnesium (4.55 ± 0.25 mEq l^{-1}), and calcium (10.4 ± 0.21 mg dl^{-1}) were all highest in fish sampled from Jones Beach (significantly higher sodium, phosphate, and magnesium than at Rockaway Beach and Sandy Hook; Figure 5). Finally, serum potassium (3.31 ± 0.08 mEq l^{-1}) was significantly lower in Atlantic sturgeon sampled from Jones Beach than at Rockaway Beach and Sandy Hook (Figure 5).

Discussion

The *D. oblongum* collected from Atlantic sturgeon here were similar in size to that of a close relative, *Anthosoma crassus*, known to cause lesions in the mouth and jaw of the shortfin mako shark (*Isurus oxyrinchus*; Benz *et al.*, 2002). *Anthosoma crassus* infestation is suggested to occur over a few months leading to neovascularization, haemorrhage, and epidermal erythrocytosis in host tissues. Based on sampling from September to November and the histological evaluation done here, a similar relationship between *D. oblongum* and the sturgeon host is indicated. It has been suggested that *A. crassus* secrete

angiogenic factors and anticoagulants to facilitate feeding and are considered capable of causing morbidity and mortality of the shark hosts they infest (Benz *et al.*, 2002). Other parasitic copepods are also known to secrete enzymes, proteases, and immunomodulatory compounds onto the surface of their hosts (Johnson and Fast, 2004; Wagner *et al.*, 2008). As *D. oblongum* appear to have similarly long associations with their hosts and do not appear to be made for efficient mobility (i.e. loss of swimming appendages and lack of dorsoventral flattening), the secretion of components to assist in localized blood feeding also seems likely. As mentioned above, gravid females were only found within the opercular cavity and attached to gills, suggesting the selection of sites more accessible to host blood than the fins or other body sites. This is a common theme in parasitic arthropods, because the production of eggs is often associated with an increased requirement for host blood (i.e. mosquitoes, ticks, ergasilid copepods, etc.). Along those lines, one may also expect, similar to the situation in *L. salmonis* and other species of sea lice, that the maturation to adult parasites (especially gravid females in this case) is accompanied by increased osmotic stress on the host organism (Jones *et al.*, 1990; Tully and Nolan, 2002; Johnson and Fast, 2004; Wagner *et al.*, 2008).
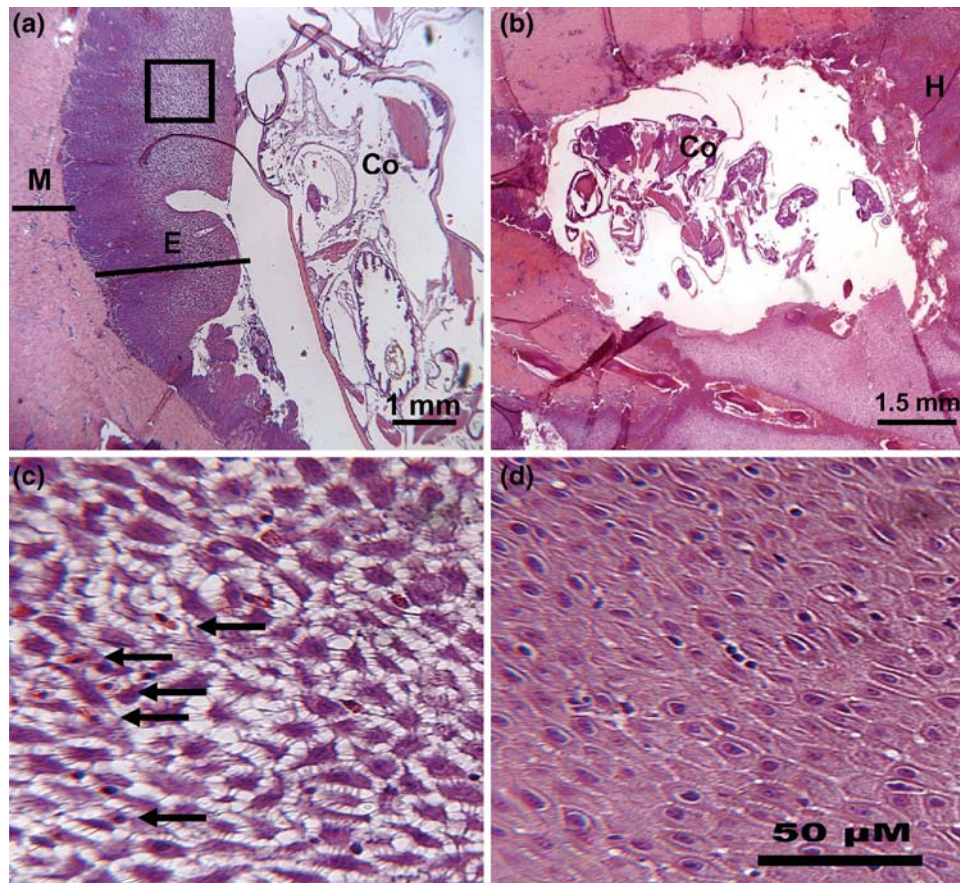
Figure 4. Atlantic sturgeon epithelium (a), (b), and (c) next to juvenile *D. oblongum* infestation, and (d) away from infestation. Co, copepod; E, epithelium; M, muscle; H, haemorrhage. The square in (a) denotes the area of higher magnification in (c). Note in (c) the intercellular oedema and erythrocyte infiltration into the epithelium on the left (arrows).

As juvenile Atlantic sturgeon inhabit estuaries highly impacted by human water usage, the effects of anthropogenic stressors on sturgeon health are of great concern. The interaction these stressors may have with other environmental conditions and how that affects osmotic competence and the ability of sturgeon to fight off the pathogenic organisms they encounter are unknown. Although there are currently no known diseases that threaten populations of Atlantic sturgeon, an epizootic (1936) of *Nitzschia sturionis* (gill monogenean) in the Aral Sea following *A. stellatus* introduction was reported to be responsible for a massive mortality of the local species of sturgeon (*A. nudiventris*), leading to a significant population decline (Zholdasova, 1997; Bauer *et al.*, 2002). Copepod parasites have also been documented to cause anaemia and impaired physiological status in sturgeon (Bauer *et al.*, 2002). In particular, these ectoparasites, which feed on gill and skin tissue, can also affect host osmotic competence through direct epithelial damage and necrosis and indirectly through elevation of host stress hormones.

To our knowledge, this is the first work on clinical serum chemistry of marine stage *A. oxyrinchus* and therefore there is little or no comparative literature. Previous work on 2-year-old freshwater *A. oxyrinchus* (Baker *et al.*, 2005) and 2-year-old *A. persicus* maintained in seawater (Kazemi *et al.*, 2006) suggests that serum sodium, calcium, and magnesium levels are all much higher in the fish sampled here. However, recently we have obtained information that the serum ion levels in Rockaway/Sandy Hook sturgeon are within the physiological ranges for seawater-adapted Atlantic sturgeon (S. McCormick, Conte Anadromous Fish Research Center, pers. comm.). Serum ion levels were also similar to those found in other diadromous fish (salmonids) during the seawater portion of their life cycle (Grimnes and Jakobsen, 1996; Bjørn and Finstad, 1997). Furthermore, glucose levels in the sturgeon sampled here by short trawls were $<30\%$ of that observed in Atlantic sturgeon caught by gillnets with longer soaking times (>1 h; MDF, pers. obs.). As glucose levels were not observed to change based on sturgeon sampling order, were within physiological resting ranges observed in other sturgeon species (Baker *et al.*, 2005; Knowles *et al.*, 2006), and were significantly lower than observed in Atlantic sturgeon undergoing extended confinement stress (gillnet; MDF, pers. obs.), we are confident that the stress related to capture did not influence the blood chemistry results.

Serum sodium, calcium, phosphate, magnesium, bicarbonate, and urea nitrogen all showed similar relationships with total *D. oblongum* abundance across sampling sites. Serum anion gap and potassium showed an inverse relationship with *D. oblongum* abundance. When data were grouped based on the level of infestation (0, 1–5, and >6 GF) rather than sampling site, the relationship remained, but the significance was lost. This suggests that although *D. oblongum* burden is an important factor in changes
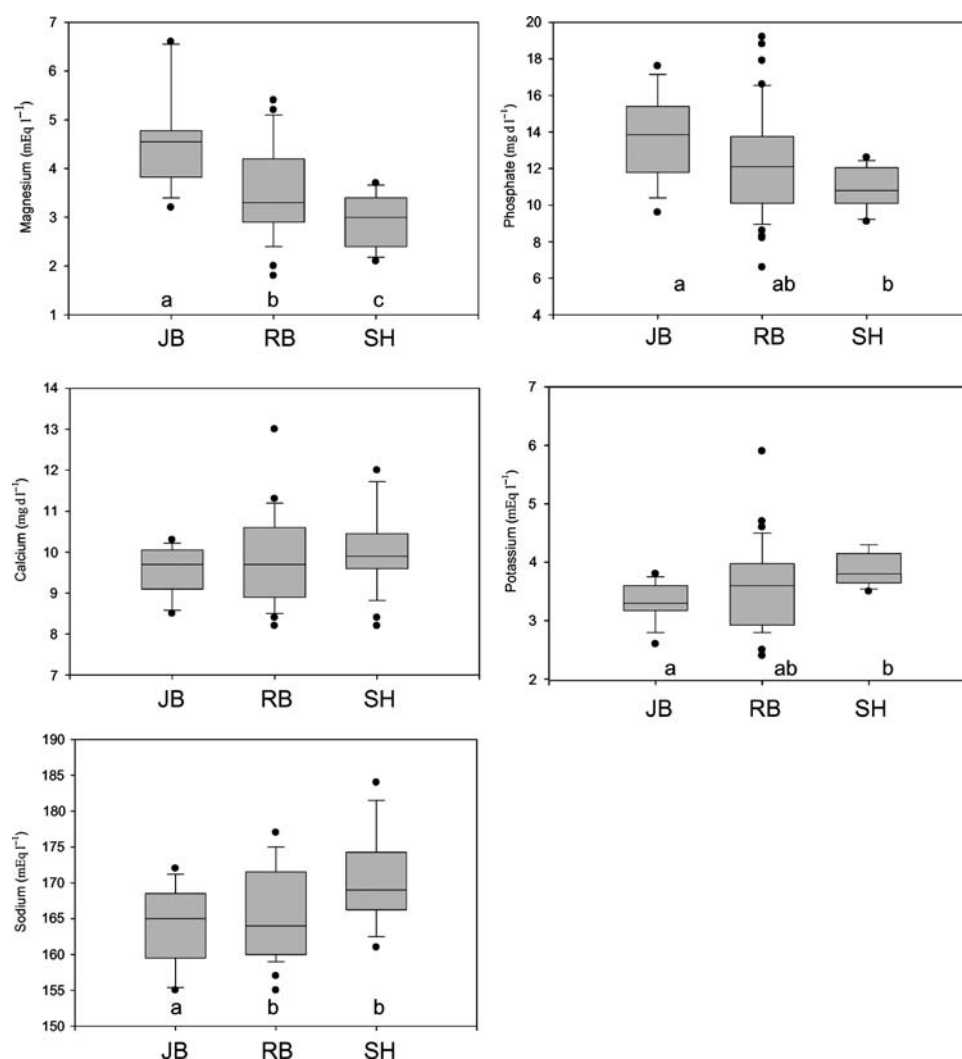
Figure 5. Median serum ion levels (10th, 25th, 75th, and 90th percentiles, and outliers) of infestation across three sampling sites in New York and New Jersey waters. JB, Jones Beach; RB, Rockaway Beach; SH, Sandy Hook, NJ. Letters denote significant difference between groups.

to serum chemistry and overall physiological robustness, in Atlantic sturgeon the sampling site may be the driving stressor that is affecting homeostatic processes.

We do not have hydrological data or extensive topographic information for these sites, but we are aware of one distinguishing feature between the Jones Beach area and the other two sampling sites. All three areas of study are close to heavily urbanized centres and can be expected to undergo some level of anthropogenic impact, but the Jones Beach area is the only one in which a sewage outfall is located. The outfall extends a few kilometres out from shore through a pipe of 213 cm diameter (Briggs, 1984). Although we do not have current data for the output of this sewage outfall, it was expected to produce $1.7 \times 10^8 \, l \, d^{-1}$ from a secondary treatment facility in Wantaugh, NY (Briggs, 1984). The effects of sewage effluent on fish physiology and immunology are well described (Iger *et al.*, 1994; Bols *et al.*, 2001; Billiard and Khan, 2003; Hogue and Peng, 2003). In the current study, significantly higher parasite burden and reduced physiological robustness at the Jones Beach site would indicate that sewage contamination at this site is negatively influencing the local population of juvenile Atlantic sturgeon.

The increased serum ion concentrations were similar to findings in Atlantic salmon (*Salmo salar*) and sea trout (*S. trutta*) infested with the parasitic copepod, *L. salmonis* (Grimnes and Jakobsen, 1996; Bjørn and Finstad, 1997). This may be a result of osmoregulatory imbalance (i.e. water loss) attributable to decreased osmotic competence from elevated cortisol, the extensive lesions and breached epithelial layer, as well as reduced function of Na^+K^+ATPase (important in NaCl regulation) in gill filaments damaged by contaminant exposure and/or parasitic copepod attachment and feeding (Grimnes and Jakobsen, 1996; Bjørn and Finstad, 1997; Nolan *et al.*, 1999; Lerner *et al.*, 2007). The lower bicarbonate concentrations and elevated anion gap were also suggestive of metabolic acidosis in heavily infested fish from Jones Beach (Kraut and Madias, 2007). This is due to a reduction in the buffering capacity of the serum through the loss of bicarbonate. Metabolic acidosis can be brought on by strenuous activity and lactic acid build-up. These data taken together suggest possible osmoregulatory stress and reduced immunological capacity in fish collected from Jones Beach. The effects of *D. oblongum* in conjunction with possible anthropogenic stress from sewage contamination may have significant ecological

impacts on juvenile Atlantic sturgeon. Determination of the temporal and spatial scales at which this impact on Atlantic sturgeon exists, as well as other possible benthic ecological impacts, warrant further investigation.

Acknowledgements

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